General

Guideline Title

Principles of analytic validation of immunohistochemical assays: guideline from the College of American Pathologists Pathology and Laboratory Quality Center.

Bibliographic Source(s)

Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, Fulton RS, Goldsmith JD, Haas TS, Karabakhtsian RG, Loykasek PA, Marolt MJ, Shen SS, Smith AT, Swanson PE. Principles of analytic validation of immunohistochemical assays: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med. 2014 Nov;138(11):1432-43. [59 references] PubMed

Guideline Status

This is the current release of the guideline.

This guideline meets NGC's 2013 (revised) inclusion criteria.

Recommendations

Major Recommendations

The grades for strength of recommendations (Strong recommendation, Recommendation, Expert consensus opinion) are defined at the end of the "Major Recommendations" field.

1. Laboratories must validate all immunohistochemical (IHC) tests before placing into clinical service. (Recommendation)
   Note: Such means include (but are not necessarily limited to):
   1. Correlating the new test's results with the morphology and expected results
   2. Comparing the new test's results with the results of prior testing of the same tissues with a validated assay in the same laboratory
   3. Comparing the new test's results with the results of testing the same tissue validation set in another laboratory using a validated assay
   4. Comparing the new test's results with previously validated non-immunohistochemical tests
   5. Testing previously graded tissue challenges from a formal proficiency testing program (if available) and comparing the results with the graded responses
2. For initial validation of every assay used clinically, with the exception of human epidermal growth factor receptor 2 (HER2/*neu*), estrogen receptor (ER), and progesterone receptor (PgR) (for which established validation guidelines already exist), laboratories should achieve at least 90% overall concordance between the new test and the comparator test or expected results. If concordance is less than 90%, laboratories need to investigate the cause of low concordance. (Recommendation)
3. For initial analytic validation of nonpredictive factor assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director determines that fewer than 20 validation cases are sufficient for a specific marker (e.g., rare antigen), the

rationale for that decision needs to be documented. (Expert consensus opinion)
Note: The validation set should include high and low expressors for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported quantitatively.

4. For initial analytic validation of all laboratory-developed predictive marker assays (with the exception of HER2/*neu*, ER, and PgR), laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented. (Expert consensus opinion)
Note: Positive cases in the validation set should span the expected range of clinical results (expression levels). This recommendation does not apply to any marker for which a separate validation guideline already exists.

5. For a marker with both predictive and nonpredictive applications, laboratories should validate it as a predictive marker if it is used as such. (Recommendation)
6. When possible, laboratories should use validation tissues that have been processed by using the same fixative and processing methods as cases that will be tested clinically. (Recommendation)
7. If IHC is regularly done on cytologic specimens that are not processed in the same manner as the tissues used for assay validation (e.g., alcohol-fixed cell blocks, air-dried smears, formalin-postfixed specimens), laboratories should test a sufficient number of such cases to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative cases and the number of predictive and nonpredictive markers to test. (Expert consensus opinion)
8. If IHC is regularly done on decalcified tissues, laboratories should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative tissues and the number of predictive and nonpredictive markers to test. (Expert consensus opinion)
9. Laboratories may use whole sections, tissue microarrays (TMAs), and/or multitissue blocks (MTBs) in their validation sets as appropriate. Whole sections should be used if TMAs/MTBs are not appropriate for the targeted antigen or if the laboratory medical director cannot confirm that the fixation and processing of TMAs/MTBs is similar to clinical specimens. (Recommendation)
10. When a new reagent lot is placed into clinical service for an existing validated assay, laboratories should confirm the assay's performance with at least 1 known positive case and 1 known negative case. (Expert consensus opinion)
11. Laboratories should confirm assay performance with at least 2 known positive and 2 known negative cases when an existing validated assay has changed in any one of the following ways:
    1. Antibody dilution
    2. Antibody vendor (same clone)
    3. Incubation or retrieval times (same method). (Expert consensus opinion)

12. Laboratories should confirm assay performance by testing a sufficient number of cases to ensure that assays consistently achieve expected results when any of the following have changed:
    1. Fixative type
    2. Antigen retrieval method (e.g., change in pH, different buffer, different heat platform)
    3. Antigen detection system
    4. Tissue processing or testing equipment
    5. Environmental conditions of testing (e.g., laboratory relocation)
    6. Laboratory water supply

    The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test. (Expert consensus opinion)

13. Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay. (Expert consensus opinion)
14. The laboratory must document all validations and verifications in compliance with regulatory and accreditation requirements. (Expert consensus opinion)

Definitions:

Grades for Strength of Evidence

Grade	Description
Convincing	Two or more level 1[a] or level 2[b] studies (study design and execution) that had an appropriate number and distribution of challenges[c] and reported consistent[d] and generalizable[d] results.

Grade	Description
Adequate	One level 1 or level 2 study that had an appropriate number and distribution of challenges and reported generalizable results. Two or more level 1 or level 2 studies that lacked the appropriate number and distribution of challenges OR were consistent but not generalizable.
Inadequate	Combinations of level 1 or level 2 studies that show unexplained inconsistencies OR one or more level 3[f] or level 4[g] studies OR expert opinion.

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[a]Level 1 study: Collaborative study using a large panel of well-characterized samples; summary data from external proficiency-testing schemes or interlaboratory comparisons.

[b]Level 2 study: High-quality peer-reviewed studies (e.g., method comparisons, validation studies).

[c]Based on number of possible response categories and required confidence in results.

[d]Consistency assessed by using central estimates/ranges or testing for result homogeneity.

[e]Generalizability is the extension of findings and conclusions from 1 study to other settings.

[f]Level 3 study: Lower-quality peer-reviewed studies OR expert panel–reviewed US Food and Drug Administration summaries.

[g]Level 4 study: Unpublished or non–peer-reviewed data.

Grades for Strength of Recommendations

Designation	Recommendation	Rationale
Strong Recommendation	*Recommend for* or *against* a particular analytic validation practice (can include must or should).	Strength of evidence is *convincing*, based on consistent, generalizable, good-quality evidence; further studies are unlikely to change the conclusions.
Recommendation	*Recommend for* or *against* a particular analytic validation practice (can include should or may).	Strength of evidence is *adequate*, based on limitations in the quality of evidence; further studies may change the conclusions.
Expert Consensus Opinion	*Recommend for* or *against* a particular analytic validation practice (can include should or may).	Important validation element to address but strength of evidence is *inadequate*; gaps in knowledge may require further studies.

Clinical Algorithm(s)

None provided

Scope

Disease/Condition(s)

Any disease or condition requiring pathological evaluation of patient specimens using immunohistochemical (IHC) assays

Guideline Category

Evaluation

Technology Assessment

Clinical Specialty

Pathology

Intended Users

Clinical Laboratory Personnel

Health Care Providers

Health Plans

Physicians

Guideline Objective(s)

- To develop recommendations for initial analytic validation and revalidation of immunohistochemical (IHC) assays
- To address the overarching question, "What is needed for initial analytic assay validation before placing any IHC test into clinical service and what are the revalidation requirements?" The scope questions are as follows:
    - When and how should validation assess analytic sensitivity, analytic specificity, accuracy (assay concordance), and precision (interrun and interoperator variability)?
    - What is the minimum number of positive and negative cases that need to be tested to analytically validate an IHC assay for its intended use(s)?
    - What parameters should be specified for the tissues used in the validation set?
    - How do certain preanalytic variables influence analytic validation?
    - What conditions require assay revalidation?

Target Population

Patients with any disease or condition requiring pathological evaluation of specimens using immunohistochemical (IHC) assays

Interventions and Practices Considered

1. Analytic validation of all immunohistochemical (IHC) tests before placing into clinical service
    - Parameters for validation including number, expression levels, fixative and processing methods
    - Performing comparative tests
2. Confirmation of assay performance when there are changes in test methods, reagents, or equipment
3. Full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay
4. Documentation all validations and verifications in compliance with regulatory and accreditation requirements

Major Outcomes Considered

- Analytic sensitivity (detection rate)
- Analytic specificity (1-false positive rate)
- Reliability (e.g., repeatability of test results)
- Assay robustness (e.g., resistance to small changes in pre-analytic or analytic variables)

Methodology

Methods Used to Collect/Select the Evidence

Hand-searches of Published Literature (Primary Sources)

Hand-searches of Published Literature (Secondary Sources)

Searches of Electronic Databases

Searches of Unpublished Data

Description of Methods Used to Collect/Select the Evidence

Literature Search and Selection

Electronic searches of the English language-published literature in Ovid MEDLINE®, U.S. National Library of Medicine PubMed, and Elsevier Scopus databases were initially conducted for the time period January 2004 to May 2012; an update was conducted through May 2013. In addition to peer-reviewed journal articles, the search identified books, book chapters, and published abstracts from English-language sources. Bibliographies of included articles were hand searched, and additional information was sought through targeted grey literature electronic searches (e.g., Google) and review of laboratory compliance and guidance Web sites (e.g., Clinical and Laboratory Standards Institute, U.S. Food and Drug Administration [FDA], National Guideline Clearinghouse [NGC], Wiley Cochrane Library).

Inclusion Criteria

Published studies were selected for full-text review if they met each of the following criteria:

- English-language articles/documents that addressed immunohistochemical (IHC) and provided data or information relevant to one or more key questions
- Study designs included validation, method comparison, cohort, or case-controlled studies, clinical trials, and systematic reviews, as well as qualitative information from consensus guidelines, regulatory documents or US and international proficiency testing reports
- Articles/documents focused on the clinical use of IHC for identification of non-FDA approved predictive and nonpredictive markers and analytic variables

Exclusion Criteria

Editorials, letters, commentaries, and invited opinions were not included in the study. Articles were also excluded if the full article was not available in English, did not address any key question, and/or focused primarily on assay optimization, quality control or quality assurance, basic or nonhuman research, nontissue immunoassays, preanalytic and postanalytic variables, or clinical validation only.

For further details on the literature search, including medical subject headings (MeSH) terms and keywords, see the supplemental digital content document (see the "Availability of Companion Documents" field).

Number of Source Documents

Of the 1463 studies identified by electronic searches, 126 met inclusion criteria and underwent data extraction. These included 122 published peer-reviewed articles, 2 book chapters, and 2 grey literature documents. Among the extracted documents, 43 did not meet minimum quality standards, presented incomplete data or data that were not in useable formats, or included only information based on expert opinion. These articles were not included in analyses or narrative summaries.

Methods Used to Assess the Quality and Strength of the Evidence

Weighting According to a Rating Scheme (Scheme Given)

Rating Scheme for the Strength of the Evidence

Grades for Strength of Evidence

Grade	Description
Convincing	Two or more level 1[a] or level 2[b] studies (study design and execution) that had an appropriate number and distribution of challenges[c] and reported consistent[d] and generalizable[d] results.

Grade	Description
Adequate	One level 1 or level 2 study that had an appropriate number and distribution of challenges and reported generalizable results. Two or more level 1 or level 2 studies that lacked the appropriate number and distribution of challenges OR were consistent but not generalizable.
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Methods Used to Analyze the Evidence

Systematic Review with Evidence Tables

Description of the Methods Used to Analyze the Evidence

Quality Assessment

Grading the quality of individual studies was performed from study design–specific criteria by the methodology consultant (L.A.B.), with input as needed from the expert panel. The aim of analytic validation is to determine a test's ability to accurately and reliably detect the antigen or marker of interest in specimens consistent with those to be tested in clinical practice. Analytic validity studies have a different design, compared to studies of diagnostic accuracy or therapeutic interventions. For this reason, the criteria needed to assess the quality of analytic validity studies are different. Quality in this context is considered to be essentially equivalent to internal validity and is assessed on the basis of study design and execution, analyses, and reporting. The strength of evidence for individual key questions or outcomes was assessed by using published criteria. The criteria included the quality and execution of studies, the quantity of data (number and size of studies), and the consistency and generalizability of the evidence across studies. Strength of evidence was graded convincing, adequate, or inadequate (see the "Rating Scheme for the Strength of the Evidence" field).

For further details on the quality assessment of evidence, including data extraction and analysis, see the supplemental digital content document (see the "Availability of Companion Documents" field).

Methods Used to Formulate the Recommendations

Expert Consensus (Nominal Group Technique)

Description of Methods Used to Formulate the Recommendations

Panel Composition

The College of American Pathologists (CAP) Pathology and Laboratory Quality Center (the Center) convened expert and advisory panels consisting of members with expertise in immunohistochemistry. Panel members included pathologists, histotechnologists, methodologists, and CAP staff. CAP approved the appointment of the project chair and panel members.

Assessing the Strength of Recommendations

Development of recommendations requires that the panel review the identified evidence and make a series of key judgments. Grades for strength of recommendations were developed by the CAP Pathology and Laboratory Quality Center and are described in the "Rating Scheme for the Strength of the Recommendations" field.

A detailed description of the methods and systematic review (including the 7 key questions, quality assessment, and complete analysis of the evidence) used to create this guideline can be found in the supplemental digital content document (see the "Availability of Companion Documents" field).

Rating Scheme for the Strength of the Recommendations

Grades for Strength of Recommendations

Designation	Recommendation	Rationale
Strong Recommendation	*Recommend for* or *against* a particular analytic validation practice (can include must or should).	Strength of evidence is *convincing*, based on consistent, generalizable, good-quality evidence; further studies are unlikely to change the conclusions.
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Cost Analysis

Formal cost analysis or cost-effectiveness was not performed.

Method of Guideline Validation

External Peer Review

Internal Peer Review

Description of Method of Guideline Validation

An independent review panel (IRP) was assembled to review the guideline and recommend approval to the College of American Pathologists (CAP). The IRP was masked to the expert panel and vetted through the conflict of interest (COI) process.

Note: For a list of external peer reviewers see the supplemental digital content document (see the "Availability of Companion Documents" field).

Evidence Supporting the Recommendations

Type of Evidence Supporting the Recommendations

The type of supporting evidence is identified and graded for each recommendation (see the "Major Recommendations" field).

Benefits/Harms of Implementing the Guideline Recommendations

Potential Benefits

The panel found that analytic validation provides a net benefit for the overall performance and safety of immunohistochemical (IHC) tests by contributing to the avoidance of potential harms related to analytic false-positive and false-negative test results.

Potential Harms

Note that there have been reports of false-positive and false-negative results due to inadequate immunohistochemical (IHC) validation.

Qualifying Statements

Qualifying Statements

The College of American Pathologists (CAP) developed the Pathology and Laboratory Quality Center as a forum to create and maintain evidence-based practice guidelines and consensus statements. Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. CAP makes no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. CAP assumes no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

Implementation of the Guideline

Description of Implementation Strategy

The College of American Pathologists (CAP) will host an Immunohistochemical (IHC) Validation Resource web page, which will include a link to manuscript and supplemental digital content, summary of recommendations, teaching PowerPoint, frequently asked questions (FAQ) document, and a free archived webinar. The guideline will be promoted and presented at various professional society meetings, including CAP, the United States and Canadian Academy of Pathology (USCAP), the National Society for Histotechnologists (NSH), the American Society of Clinical Pathology (ASCP), and the American Society of Cytopathology (ASC).

Implementation Tools

Quick Reference Guides/Physician Guides

Slide Presentation

For information about availability, see the *Availability of Companion Documents* and *Patient Resources* fields below.

Institute of Medicine (IOM) National Healthcare Quality Report Categories

IOM Care Need

Staying Healthy

IOM Domain

Effectiveness

Identifying Information and Availability

Bibliographic Source(s)

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Adaptation

Not applicable: The guideline was not adapted from another source.

Date Released

2014 Nov

Guideline Developer(s)

College of American Pathologists - Medical Specialty Society

Source(s) of Funding

The College of American Pathologists (CAP) provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement.

Guideline Committee

College of American Pathologists (CAP) Pathology and Laboratory Quality Center Expert and Advisory Panel

Composition of Group That Authored the Guideline

Panel Members: Patrick L. Fitzgibbons, MD, Department of Pathology, St. Jude Medical Center, Fullerton, California; Linda A. Bradley, PhD, Department of Pathology and Laboratory Medicine, Women & Infants Hospital/Brown University, Providence, Rhode Island; Lisa A. Fatheree, BS, SCT(ASCP), College of American Pathologists, Northfield, Illinois; Randa Alsabeh, MD, Department of Pathology, Kaiser Permanente - Los Angeles Medical Center, Los Angeles, California; Regan S. Fulton, MD, PhD, PhenoPath Laboratories, Seattle, Washington; Jeffrey D. Goldsmith, MD, Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; Thomas S. Haas, DO, Department of Pathology, Mercy Hospital, Janesville, Wisconsin; Rouzan G. Karabakhtsian, MD, PhD, Department of Pathology, Montefiore Medical Center, New York, New York; Patti A. Loykasek, HT(ASCP), Regional Medical Laboratory, St John's Medical Center, Tulsa, Oklahoma; Monna J. Marolt, MD, Department of Pathology, University of Minnesota Medical Center, Fairview, Minneapolis; Steven S. Shen, MD, PhD, Department of Pathology, The Methodist Hospital, Houston, Texas; Anthony T. Smith, MLS, College of American Pathologists, Northfield, Illinois; Paul E. Swanson, MD, Department of Pathology, University of Washington Medical Center, Seattle, Washington

Financial Disclosures/Conflicts of Interest

Before acceptance on the expert or advisory panel, potential members completed the College of American Pathologists (CAP) conflict of interest disclosure process, whose policy and form (in effect April 2010) require disclosure of material financial interest in or potential for benefit of significant value from the guideline's development or its recommendations 12 months prior through the time of publication. Potential members completed the conflict of interest disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Everyone was required to disclose conflicts before beginning and continuously throughout the project's timeline. One expert panel member (R.S.F.) was recused from discussion and voting on the recommendation pertaining to tissue microarrays, and one (T.S.H.) was recused from voting on recommendations pertaining to potential increased antibody usage. Expert panel members' disclosed conflicts are listed in the Appendix of the original guideline document. Please see the supplemental digital content for full details on the conflict of interest policy (see the "Availability of Companion Documents" field).

Guideline Status

This is the current release of the guideline.

This guideline meets NGC's 2013 (revised) inclusion criteria.

Guideline Availability

Electronic copies: Available from the Archives of Pathology & Laboratory Medicine Journal Web site.

Availability of Companion Documents

The following are available:

- Principles of analytic validation of immunohistochemical assays. Summary of recommendations. 2014. 2 p. Electronic copies: Available from the College of American Pathologists (CAP) Web site.
- Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, Fulton RS, Goldsmith JD, Haas TS, Karabakhtsian RG, Loykasek PA, Marolt MJ, Shen SS, Smith AT, Swanson PE. Principles of analytic validation of immunohistochemical assays: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Supplemental digital content. 2014. 44 p. Electronic copies: Available from the CAP Web site.
- Principles of analytic validation of immunohistochemical assays. Frequently asked questions. 2014 Apr. 4 p. Electronic copies: Available from the CAP Web site.
- Principles of analytic validation of immunohistochemical assays. Slide presentation. 2014 Mar. 57 p. Electronic copies: Available from the CAP Web site.

Patient Resources

None available

NGC Status

This NGC summary was completed by ECRI Institute on December 11, 2014. The information was verified by the guideline developer on January 22, 2015.

Copyright Statement

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